SURFACE MODIFICATION FOR BIOCOMPATIBILITY

Contract No. NS 5-2322

Quarterly Progress Report #3

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The University of Michigan

David C. Martin and K. Sue O'Shea

Quarterly Progress to:	National Institute of Health
Contract Monitor:	William Heetderks, Ph.D.
Research Contract	"Surface Modification for Biocompatibility"
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Principal Investigators:	David C. Martin and K. Sue O'Shea
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Overview

This report is a summary of our activity in the third quarter of 1995. The major event of this period was our participation in the annual Neural Prosthesis program at the NIH, where we were able to meet with others involved in this program. This was a very rewarding experience, and we continue to follow up with developments based on these interactions.

In the third period this year we continued our efforts to deposit and characterize microstucturally controlled bioactive protein polymer films on solid surfaces and their biological activity in vivo. We have also begun efforts to obtain quantitative information about the electronic properties of the coatings, in collaboration with Prof. David Anderson's group (Jim Weiland) in the EECS department.

We have also obtained data on the *in vivo* performance of these materials when implanted into the Central Nervous System (CNS). This report provides an overview of the major results to date and discusses our plans for the future. We have been working to evaluate (1) protein polymer film deposition and morphology, (2) bioactivity of protein polymer films *in vitro*, and (3) bioactivity and stability of protein polymer films *in vivo*. We also describe our efforts to discuss our work in (4) external communications with the scientific community.

1. Protein Film Deposition and Morphology

Progress:

We have now completed an extended range of investigations to explore the effect of protein polymer coverage on solid substrates and the biological response. These data are shown in the enclosed figure, which plots the number of attached and spread cells as a function of solution concentration of protein. Also shown on the plot are values for the film thickness and percent of surface coverage, as estimated by atomic force microscopy (AFM).

We also confirmed that information about the electrical properties of the coated probes can be obtained by impedence spectroscopy. We were able to run some sample probes on instrumentation in Prof. Meyerhoff's laboratory in the Chemistry department, observed clear differences in electronic performance between coated and uncoated probes. The impedence was higher at low frequencies in the coated than in the uncoated probes, as has been observed in other cases such as thin Langmuir-Blodgett films. Detailed results are being tabulated and will be reported in the near future.

Plans:

We are now starting systematic studies of probe electrical performance as a function of coating thickness and morphology. We are also continuing our detailed studies of protein film morphology by low voltage scanning electron microscopy, atomic force microscopy, and transmission electron microscopy.

2. Bioactivity of Protein Polymer Films *in vitro*

Progress:

Additional studies to reveal the biological response of the protein films described in our first progress report are continuing to provide information about cellular adhesion as a function of protein coverage. These studies involve quantitative evalution of cell adhesion and spreading on protein polymer coated surfaces.

PC12 (pheochromocytoma) cells are derived from the adrenal medulla, and differentiate into a relatively pure population of neurons in response to nerve growth factor (NGF) (50 ng/ml). We have now conducted experiments that confirm that growth factors suspended in polymer coatings can be delivered to target neurons. NGF was suspended in protein polymers and used to coat coverslips on which target PC12 cells were grown for periods of 1-5 days in vitro. These experiments showed that the growth factor remained active and available to cells after processing.

To determine whether it is possible to deliver a growth factor via the polymer, nerve growth factor (0.1 mg) was dissolved in water, then rapidly mixed with 0.1 ml SLPL dissolved in formic acid (final NGF concentration appx. 20 ng/ml). The polymer/growth factor blend (0.03 % NGF by weight) was electrospun onto glass coverslips which were washed, then briefly UV light sterilized.

Pheochromocytoma cells are derived from a tumor of the rat adrenal medulla; when maintained in vitro they exhibit growth characteristics of a transformed cell line--they proliferate rapidly, and are not highly differentiated. When grown in the presence of nerve growth factor (NGF; 50 ng/ml for periods of 3-5 days), they differentiate into neuronal cells. On appropriate substrates (e.g., laminin) they exhibit extensive neurite outgrowth. In order to determine if the NGF/SLPF was biologically active, clonal PC-12 cells were plated at 5 x 10^5 cells/ml onto NGF/SLPF coverslips. Additional controls were grown on SLPF, on SLPF to which 50 ng/ml NGF was added. After periods of 4-5 days on NGF/SLPF PC-12 cells extended long neurites, quite similar to those seen on SLPF to which exogenous NGF was added. On SLPF alone there was no differentiation of PC-12 into a neuronal morphology. Future experiments will involve employing a higher concentration of NGF, examining a possibly synergistic effect of NGF with SLPL (rather than SLPF) coated substrates, and coating suture for implantation. It might be argued that the NGF became available to the cells by solubilization from the spun fiber rather than being locally available. We believe that this is not the case, as cells at the edge of the coverslip (attached to the bottom of the well) did not adopt a neuronal morphology. In any case, it will be possible to test this directly by growing PC-12 cells on a millicell insert over coverslips coated with SLPF/NGF.

3. Bioactivity of Protein Polymer Films *in vivo*

Progress:

Polypropylene suture (~50 micron diameter) was coated with the following materials and implanted in the Guinea Pig CNS:

1. no coating (control)
2. SLPF coated
3. SLPL coated
4. SLPF/Schwann cells
5. SLPF coated and exposed to CSF
6. SELP coated

The implants were in place for periods of 3 and 12 weeks. The brains were removed, blocked, and evaluated by light and electron microscopy for information about tissue response, adherence of the Schwann cell layer, and stability of the protein film. The response of the tissue near coated micromachined probes will also be evaluated using laser confocal microscopy.

Both short term (3 week) and long term (12 week) samples have now been evaluated histologically. The sample preparation involves embedding the tissue in poly(methyl methacrylate), sectioning with a stainless steel knife, and examination by optical microscopy. This effort has been conducted in collaboration with Rick Altschuler and coworkers of the Kresge Hearing Research Institute. Results to date show there is very minimal response to the implants by astrocytes, and the morphology between the three week and three months implants is similar. When the final six brains have been sectioned, we will be carrying out quantitative image analysis to construct cumulative fall of curves of astrocyte, neuronal, and microglial behavior relative to the implant.

Plans:

An additional set of three animals are being run with identical conditions to confirm and corroborate the results from this first study, and to explore the behavior of silicon probes coated with similar materials.

4. External Communications

A talk on "Surface Modification for Biocompatibility" by David C. Martin, J. Philip Anderson, Chris Buchko, K. Sue O'Shea, and Atisa Sioshansi was given at the Neural Prosthesis conference on October 20 at the NIH in Bethesda, MD.

An preprint titlted "Electric Field Mediated Deposition of Bioactive Polypeptides on Neural Prosthetic Devices", by Chris Buchko, Ken Kozloff, Atisa Sioshansi, K. Sue O'Shea, and David C. Martin is being prepared for a proceedings volumer for the fall 1995 Materials Research Society meeting in Boston, MA.

A poster titled "Glial and Neuronal Cell Response to Patterned Substrates Coated with Silk Polymers Containing Elastin, Fibronectin, or Laminin Cell Binding Motifs", by K. Sue O'Shea, Atisa Sioshansi, Chris Buchko, Joe Cappello, and David C. Martin was presented at the the Society for Neuroscience meeting.

Work continues on an invited review paper on *Processing and Characterization of Silk-like Protein Polymers*, by David C. Martin, J. Philip Anderson, Chris Buchko, and Tao Jiang, which is scheduled to appear in a special volume on *Protein Polymer Materials*, edited by Kevin McGrath and David Kaplan of the U. S. Army Natick RD&E Center. This document is scheduled to be completed on December 1 of this year.


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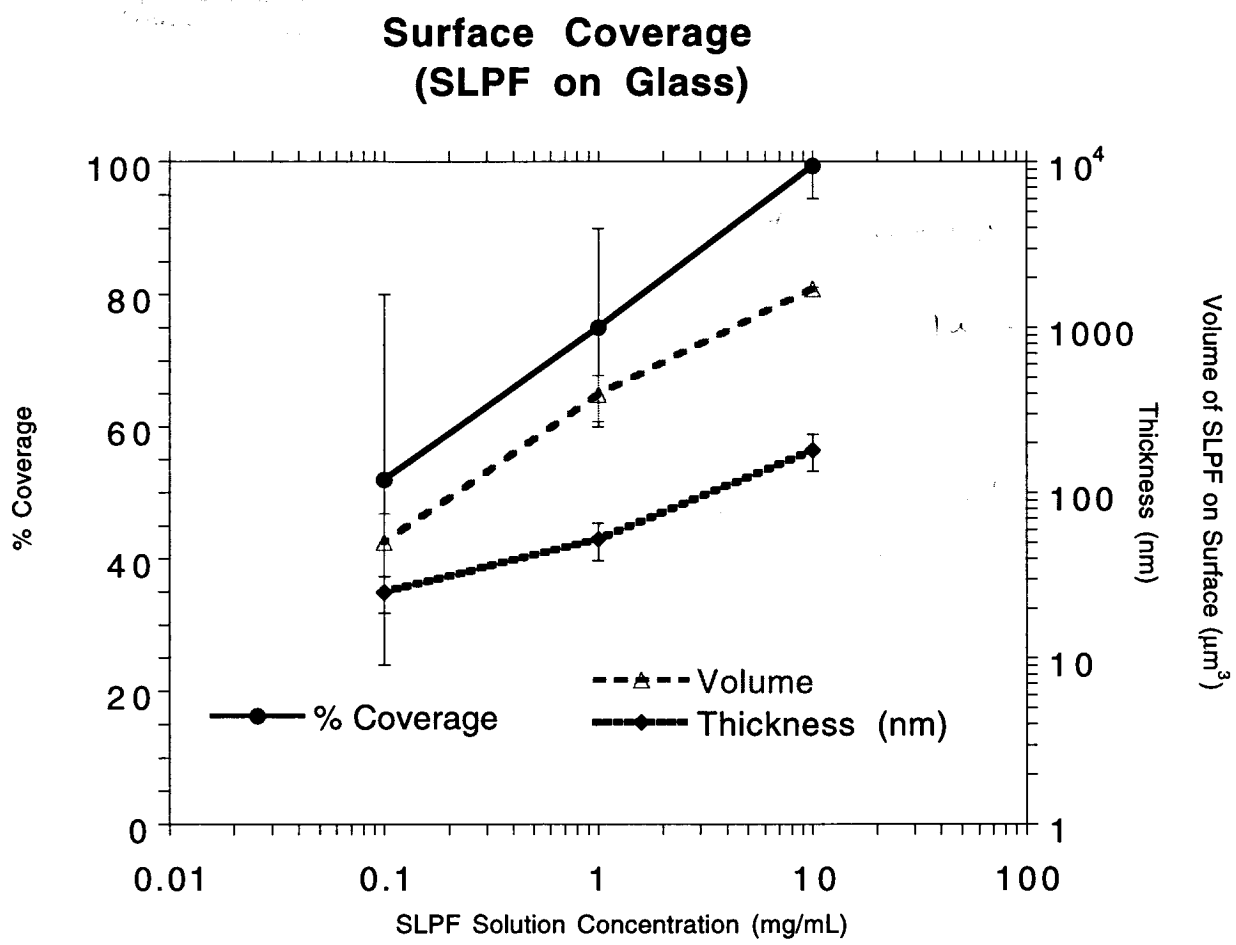
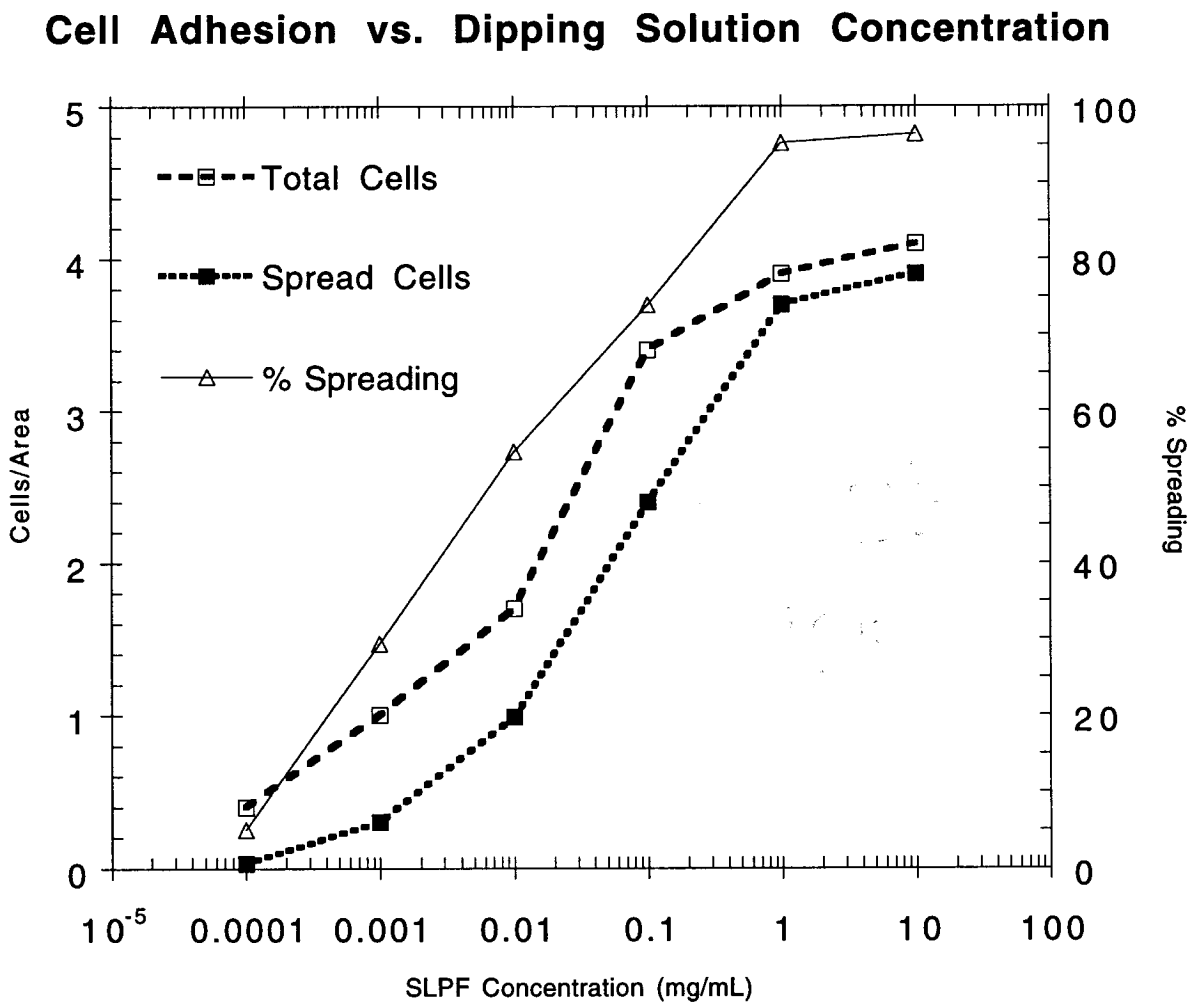

Surface Coverage
(SLPF on Glass)
% Coverage
100
80
60
40
20
0
0.01
0.1
1
10
100
SLPF Solution Concentration (mg/mL)
10^4
1000
100
10
1
Thickness (nm)
Volume of SLPF on Surface (μm^3)
Volume
% Coverage
Thickness (nm)


Cell Adhesion vs. Dipping Solution Concentration
Total Cells
Spread Cells
% Spreading
Cells/Area
% Spreading
SLPF Concentration (mg/mL)
0
1
2
3
4
5
0
20
40
60
80
100
10-5
0.0001
0.001
0.01
0.1
1
10
100